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Nanobiotechnology in the Management of Glaucoma*

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ABSTRACT

As the prevalence of glaucoma continues to rise, clinicians and researchers are confronted with an age-old problem: how to reduce risk factors and preserve vision in glaucoma. Current management options revolve around a validated paradigm—intraocular pressure reduction. Active investigations to improve drug delivery efficacy and surgical outcomes are flourishing. This article aims to provide the interested readers with a review of recent discoveries in nanobiotechnology for the management of glaucoma. Targeted drug-delivery systems using mesoscale vectors demonstrate promising delivery profiles. The utility of nanoparticulate therapies to support retinal ganglion cell survival is being investigated. Studies to modulate tissue regeneration and remodeling and improve post-trabeculectomy outcomes are underway. Though these modalities promise new avenues to manage glaucoma, immediate market availability is not anticipated soon.

Keywords: Glaucoma; Nanotechnology; Nanomedicine; Drug Delivery; Wound Healing; Nanoparticle

1. Introduction

Glaucoma is a group of diseases having characteristic optic neuropathy with associated visual function deficits. Early disease detection and vision preservation constitute the management of glaucoma. Currently, therapeutic strategies involve medical and surgical reduction of intraocular pressure (IOP). However, maintaining local therapeutic bioavailability of hypotensive agents remains a challenge in the field of ophthalmic drug delivery. Surgical modalities are effective but have associated complications [1]. Given that 60 million people worldwide are afflicted by this disease [2] and 27% of those afflicted are at risk of developing glaucoma-related blindness in one eye after 20 years [3], the demand for viable treatment alternatives, particularly improved drug delivery models, is self-evident.

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2. Nanobiotechnologies for Glaucoma Management

Previously, many clinical trials demonstrated that initial medical management is an effective option for IOP reduction and vision preservation [4-11]. Although topical instillation is the predominant route, ensuring effective local concentration is a fundamental challenge. Precorneal factors such as small load, poor tissue penetration, mechanical removal by tears and blinking, and nasolacrimal elimination prevent the medication from reaching a sustained therapeutic level and necessitate multiple dosings per day, ultimately, leading to poor patient adherence [12-14]. Other studies further report that many patients waste much of the topical medications because of incorrect instillation techniques [13-15]. Systemic absorption may precipitate adverse effects [16-21]. Therefore, efforts are directed toward developing better drug delivery systems, e.g. ophthalmic inserts, smart hydrogel, vesicular and particulate carrier systems.

Nanoscale drug delivery systems offer the therapeutic advantages of targeted tissue penetration, enhanced release kinetics, and increased local biodistribution [22-24]. In the field of tissue engineering and regenerative medi-

cine, the biologic length scale of nanostructures promotes meaningful interactions for tissue repair and regeneration [23]. “Nano” was originally defined for the semiconductor industry as having at least one dimension less than 100 nanometer; but the definition of “nano-” has been recast in an operational fashion and many authors use “nano-” to include biologically relevant length scale, such as larger macromolecules and organelles for the fields of nanobiotechnology and nanomedicine [22-24]. Below, we summarize the recent discoveries in nanobiotechnology for the management of glaucoma.

2.1. Drug Delivery Systems

2.1.1. Vesicular Platforms—Liposomes, Discomes, and Niosomes

Modeled after biologic organelles, liposomal systems are designed for encapsulation of therapeutic agents for drug delivery and have found to have increased efficacy and decreased toxicity. Their diverse systemic applications encompasses liposomal daunorubicin for Kaposi’s sarcoma, micellar paclitaxel for ovarian and breast cancer, and liposomal amphotericin B for systemic fungal infections or leishmaniasis. Surface modifications, such as PEGylation of the liposome, extend systemic circulating time and reduce toxicity.

Liposomal preparations of topical ophthalmic medications are particularly well studied for ocular surface infections. Use of liposomal amphotericin B for the treatment of fungal keratitis has been reported to significantly reduce toxicity compared to non-liposomal formulation [25,26]. Topical liposomal acyclovir shows better delivery than commercial acyclovir ointment in *in vitro* studies and animal models [27]. Liposomal formulations of gatifloxacin, ciprofloxacin and fluconazole have shown prolonged delivery profiles, leading to better *in vitro* transcorneal permeation compared to aqueous formulations [28-30].

Other investigators are evaluating the feasibility of liposome-encapsulated ocular antihypertensive drugs. Topical liposomal and niosomal formulations of acetazolamide, a carbonic anhydrase inhibitor, are being investigated for their ability to overcome acetazolamide’s limited aqueous solubility and improve its corneal permeation [31-34]. *In vitro* and *in vivo* studies demonstrate sustained release kinetics with good intraocular hypotensive effect [32].

In addition to liposomes, other vesicular platforms, such as discomes and niosomes, are also being explored for ocular antihypertensive medications. Discomes are non-ionic surfactant-based discoidal vesicles, which also improve drug delivery. Some authors have reported that discoidal vesicles containing timolol maleate produce a sustained activity profile upon introduction to the ocular cavity [35]. Niosomes are another non-ionic bilayered vesicle that can entrap both hydrophilic and lipophilic drugs, either in the aqueous layer or in the vesicular membrane. Compared to liposomes, niosomes offer some advantages, such as chemical stability and lower production cost. A recent study reported that a mucoadhesive-coated niosomal system for timolol maleate can achieve higher peak concentrations for an extended period compared to the conventional dose form [36]. Sizes of these discomes and niosomes are in the micron range [35,36]. Lastly, a more recent nanovesicular formulation of brimonidine tartrate has been constructed using sorbitan stearate and cholesterol, and the authors report significant intraocular pressure-lowering activity for a prolonged period of time compared to the commercial preparation [37-39]. Most of the above studies report no short-term toxicity to the ocular surface of the animal models.

2.1.2. Nanoparticulate Platforms

Development of nanoparticulate platforms for drug delivery has gained significant traction over recent years. The nanoscale size range and surface functionalization offer the advantages of targeted delivery and resistance to degradation. Particulate delivery systems also provide more stable storage media, compared to vesicular systems. Biodegradable polymeric platforms present an exciting opportunity for investigators, where the pharmacokinetics is determined by the particulate size and carrier material, which is ultimately controlled by synthesis technologies.

Biodegradable poly(lactic-co-glycolic) acid (PLGA) polymers have promise as potential couriers for antihypertensive agents. Timolol maleate integrated into PLGA polymers via a solvent evaporation method has been reported to have sustained release kinetics [40]. The release mechanism is proposed to be polymeric degradation, rather than microporous diffusion. Another study suggests that PLGA and poly(l-lactide) acid (PLLA) micro- and nanoparticles can deliver timolol maleate continually over a 3-month period [41]. Intravitreal injection of PLGA is safe in a rabbit model, where only a localized foreign body reaction is reported [42]. In this study, the authors observed that the choroid and retina maintain normal appearence and no clinical inflammation was detected. Accordingly, some investigators are optimistic that these biodegradable polymeric micro- and nanospheres may find application as subconjunctival depots to improve patient adherence [41]. Similarly, nanoparticulate platforms for carbonic anhydrase inhibitors are being investigated as well. Methazolamide nanoparticles are reported to have longer and higher therapeutic efficacy compared to suspension formulation and commercial eye drops [43,44].

Another polymer of ophthalmic interest is chitosan, a polycationic biodegradable polymer composed of repeating glucosamine units. The chitosan nanoparticles are thought to have longer precorneal residence time and increased corneal penetration. Gatifloxacin-loaded chitosan nanoparticles show sustained released kinetics [45]. Chitosan/polylactic acid nanoparticles containing rapamycin demonstrate improved corneal allograft survival in a rabbit model compared to aqueous suspension [46]. With respect to ocular anti-hypertensive agents, nanoparticulate formulations of chitosan and timolol maleate or dorzolamide hydrochloride have also been described [47]. These investigators find that modification of these nanoparticles with hyaluronic acid, which improves mucoadhesion, significantly decreases intraocular pressure in a rabbit model, compared to plain solutions of these drugs.

2.1.3. Dendrimeric Platforms

Dendrimers comprise a fascinating drug carrier system. These hyperbranched globular macromolecules with dendritic subunits have modifiable properties depending on their subunits and surface terminal groups. Therapeutic agents can either be encapsulated in the core of the dendrimer scaffold or conjugated on the surface. The dendrimer diameter can be customized based on its generation and terminal groups.

Some therapeutic agents being investigated include propranolol, sulfasalazine, folic acid, adriamycin, methotrexate, paclitaxel, and penicillin. Photodynamic therapy and gene therapy using dendrimers for corneal, retinal, and choroidal neovascularization have been published [48-50]. For glaucoma, poly(amidoamine) dendrimeric constructs have been constructed to deliver pilocarpine and tropicamide [51]. These dendrimers appear to have greater corneal residence time, increased bioavailability, and low ocular irritation index in animal model. A recent study reported increased uptake of timolol maleate and brimonidine using poly(amidoamine) dendrimer hydrogel as delivery modality in bovine corneal model [52].

2.2. Tissue Protection

Currently, IOP control drives glaucoma management. However, some investigators are evaluating the feasibility of neuroprotection as well. Apoptosis of retinal ganglion cells (RGC) has been associated with glaucomatous optic neuropathy [53]. Studies using RGC death after optic nerve injury in an animal model suggest that neurotrophic factors, e.g. brain-derived growth factors, insulin-like growth factors, glial-derived neurotrophic factor, and ciliary neurotrophic factors (CNTF) may support RGC survival [54-56]. Accordingly, the roles of neurotrophic factors in neuroprotection in glaucoma are being explored. Lentiviral-mediated transfer of CNTF into Schwann cells for optic nerve repair has been found to significantly increase RGC survival in animal models [57]. Others investigators have used biodegradable PLGA polymers to construct nano- and microspheres for CNTF encapsulation [58]. These authors report bioactivity in an *in vitro* neural stem cell model and note that the process of protein encapsulation with the polymer did not reduce its potency. Others incorporate CNTF nanospheres into photopolymerizable hydrogel to create a tissue engineering scaffold with intrinsic sustained drug delivery capability [59]. This scaffold provides an enhanced substratum for neural tissue repair and regeneration. Recently, induction of heat shock protein for neuroprotection using superparamagnetic nanoparticles has also been reported [60]. In the near future, successful commercial deployment of these and other developments will add another set of tools in the armamentarium of the glaucoma specialist to preserve vision.

2.3. Modulation of Postsurgical Wound Healing in Filtration Surgery

Successful glaucoma filtering surgery necessitates adequate passage of the aqueous humor from the anterior chamber to an extraocular reservoir. Exuberant cicatricial changes to the conjunctiva following glaucoma filtration procedures present a threat to long-term success [61,62]. Antifibrotic agents have been demonstrated to promote longer bleb survival but are associated with severe complications such as leakage, infection, hypotony, and endophthalmitis [63-65]. Currently, antifibrotic agents are mostly frequently introduced intraoperatively via a soaked sponge. As with drug delivery of intraocular pressure lowering medications, nanobiotechnology can be directed toward improvement of bleb survival.

A disc carrying biodegradable poly(lactic acid) microspheres loaded with 5-fluorouracil for subconjunctival implant has been developed and tested in a rabbit model [66]. The authors show that delivery of 5-fluorouracil using the carrier resulted in greater decrease in intraocular pressure, prolonged bleb persistence, and less corneal toxicity. Syntheses of various nanoparticulate 5-fluorouracil and mitomycin have also been reported [67-69]. However, ophthalmic applications of these nanoparticles have yet to be investigated.

Other investigators target the growth factor mediated cellular proliferation. In one study, glucosamine and glucosamine 6-sulfate dendrimers were used to target fibroblast growth factor-2 mediated endothelial cell proliferation and neoangiogenesis in a rabbit model of scar tissue formation after glaucoma filtration surgery [70]. The authors report that postoperative day 30 bleb survival improves from 30% to 80%. Histologic examination revealed less cicatricial proliferation in the den-

drimer group. In another approach, biodegradable porous PLGA microspheres containing antisense-TGF-β2 oligonucleotide nanocomplexes have been found to promote bleb survival in a rabbit model of filtering surgery [71]. The antisense oligonucleotide reduces synthesis of the cytokine TGF-β2, which promotes wound healing. In this study, the nanocomplexes are encapsulated into porous microspheres and administered subconjunctivally. The authors report increased penetration of the encapsulated oligonucleotides in conjunctival cells and increased time to filtering bleb failure. Steroids may also be packaged via nanoparticles to modulate postoperative wound healing. Dexamethasone entrapped in biodegradable poly(d,l-lactide-co-glycolide) (PLGA) nanoparticles has been reported [72]. These nanoparticles can find applications both in preventing filtering bleb scarring and in the investigation of steroid response in glaucoma.

Viral vectors constitute another form of particulate nanoparticles that can be directed toward glaucoma surgical management. Adenovirus-mediated gene therapy has been performed to prevent bleb scarring in a rabbit model of glaucoma filtration surgery [73]. The authors find that topical, intraoperative application of recombinant adenovirus containing the human p21 gene demonstrates inhibition of wound healing and fibroproliferation after filtration surgery, comparable to mitomycin but with fewer adverse effects. Similar results are seen with an Ad-p27 vector [74]. Interestingly, attempts to use viral vectors to blunt steroid response are also underway. Novel glucocorticoid-inducible adenovirus vectors have been developed to overproduce metalloprotein 1, which degrades collagen type I after specific activation by dexamethasone [75]. Therefore, patients who presumably need long-term steroid treatment may benefit by having increased metalloproteinase activity to aid trabecular flow while on steroids.

3. Conclusion

This brief review highlights the recent advances and advantages of nanobiotechnology for improving our understanding of glaucoma and its management. In essence, nanobiotechnology offers the potential for more effective delivery of pharmaceutical agents that can influence both the medical and surgical arms of glaucoma management.

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